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Lab resource: Stem cell line

Derivation of Genea043 human embryonic stem cell line

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ABSTRACT

The Genea043 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, through ICM outgrowth on inactivated human feeders. The line showed pluripotent cell morphology and genomic analysis verified a 46, XY karyotype and male allele pattern through traditional karyotyping, CGH and STR analysis. Pluripotency of Genea043 was demonstrated with 92% of cells expressing Nanog, 95% Oct4, 61% Tra1–60 and 99% SSEA4, a PluriTest Pluripotency score of 31.74, Novelty score of 1.2 and Alkaline Phosphatase activity. The cell line was negative for Mycoplasma and any visible contamination.

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1. Resource table

Name of stem cell line	Genea043 (Alternate ID: SIVF043)
Institution	Genea biocells
Person who created resource	Biljana Dumevska
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	March, 2009
Origin	Human embryos
Type of resource	Derived human embryonic stem cell line
Sub-type	Human pluripotent cell line
Key marker expression	Nanog, Oct4, Tra1–60, and SSEA4
Authentication	Identity and purity of cell line confirmed (Figures 1–2, Tables 1–2 below)
Link to related literature (direct URL links and full references)	(Laurent et al., 2011) http://www.ncbi.nlm.nih.gov/pubmed/?term=21211785
Information in public databases	National Institutes of Health (NIH) registered NIHhESC-13-0232 UK Stem Cell Bank (UKSCB) registered SCSC14–40 SNP Data GEO accession numbers GSM638445
Ethical approval	Obtained from the Genea Ethics Committee on 21 February 2001 under the Australian National Health and Medical Research Council (NHMRC) licence 309703

2. Resource Details

Date of derivation	February 2009
Karyotype	46, XY — no abnormalities detected
Sex	Male
Pluripotent	YES — by Alkaline Phosphatase stain positivity, Nanog, Oct4, Tra1–60, and SSEA4 staining and PluriTest
Disease status	Unaffected
Sterility	The cell line is tested and found negative for Mycoplasma and any visible contamination
Sibling lines available	YES — GENEA042 (XX NIHhESC-13-0231, SCSC14–40)

3. Materials and methods

3.1. Cell line derivation

The zona pellucida of a blastocyst-stage human embryo was manually removed using a small blade. The embryo was bisected and both ICM and trophectoderm were plated onto mitomycin C inactivated Detroit 510 HFF human feeders (plated 90,000 cells 1 well of 4 well — 47,368 cells/cm2) in 20% Knock out serum in standard hESC culture medium (Amit et al., 2000) with 20 ng/mL Fgf2. CGH, karyotyping and STR profiling was performed at the first cryobanking step from ICM outgrowths maintained on feeders. Alkaline Phosphatase staining was performed on feeders. Cells were then enzymatically passaged as single cells in M2 pluripotent cell maintenance medium (Genea Biocells) and genetic analysis repeated, immunofluorescent pluripotent marker staining, PluriTest, teratoma and sterility testing performed.

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3.2. Genetic analysis

1. Karyotyping: Passage 4; For enrichment of metaphase cells, cellular outgrowths were incubated with either 0.22 ng/ml colcemid (Invitrogen) and 37.5 g/ml BrdU (Sigma) for 17–19 h or 5 ng/ml colcemid for 2.5 h. Single cells were subsequently obtained using Non-Enzymatic Cell Dissociation Solution (Sigma) and transferred to Sydney IVF's (now Genea) diagnostic cytogenetics laboratory for preparation of metaphase spreads and cytogenetic analysis by G-banding. A minimum of 15 metaphase cells were examined, of which a full karyotype or chromosome counts was performed for 5 and 10 of the metaphases respectively.
2. Comparative Genomic Hybridization (CGH) based chromosomal analysis: Passage 10 (8 on feeders, 2 enzymatic); CGH was used to screen targeted regions of the genome for gains and losses associated with chromosomal imbalances such as aneuploidy, deletions and duplications. CGH was performed using SurePrint G3 microarrays (8 × 60 K format) which were scanned with the Agilent Scanner C and analysed using Genomic Workbench Standard Edition 5.0 software (Agilent Technologies).
3. DNA Profiling: Passage 3; DNA 'fingerprinting' was performed using the AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems #4322288) to provide permanent genetic identification of the cell lines https://www.thermofisher.com/order/catalog/product/4322288.

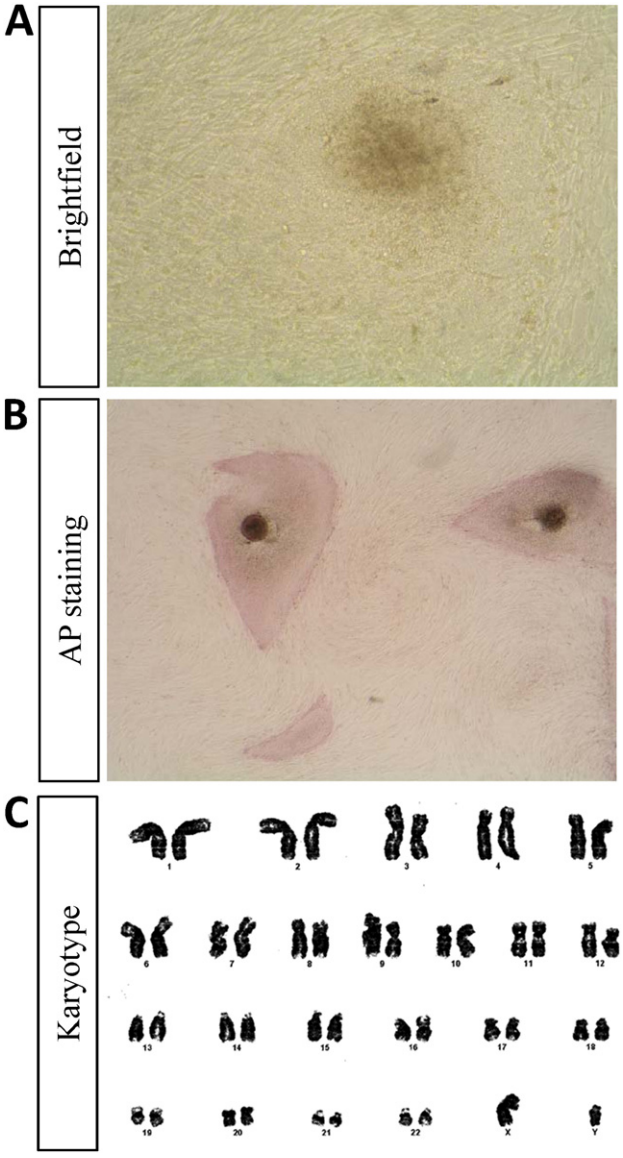

Fig. 1. Morphology and karyotype of Genea043. A) Brightfield (passage 1) growing on human inactivated feeders. B) Alkaline phosphatase activity (passage 4) on human inactivated feeders. C) Karyotypic analysis passage 4 showing 46, XY normal, male karyotype.

3.3. Pluripotency assessment

1. Alkaline Phosphatase: Passage 4; Genea043 grown on feeders were stained as per manufacturers protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004).
2. Immunofluorescence: Passage 10 (8 on feeders, 2 enzymatic); cells were fixed with formalin and stained with Nanog #560,483 1:200; Oct4 #560,217 1:150: Tra1–60 #560,121 1:150; SSEA4 #560,308 1:200 (all BD Pharmingen:). Images were acquired with an IN Cell Analyser 6000 and quantified using In Cell Developer Software (GE).
3. PluriTest: Passage 11 (8 on feeders, 3 enzymatic); RNA was collected and subjected to a *PluriTest*, a bioinformatics assay of pluripotency in human cells based on gene expression profiles (Müller et al., 2012).

3.4. Sterility testing

1. Mycoplasma: Passage 13; testing was performed at the Victorian Infectious Diseases Reference Laboratory using Mycoplasma Genus PCR.
2. Microbial contamination: Testing was performed in conjunction with our QC measures. Cells were thawed and cultured in 7 mL antibiotic free medium (Genea Biocells M2 medium) for 2–3 days at 37 °C. A clear solution at ~48–72 h indicated lack of bacterial, fungal or yeast contamination. Clarity of the solution was assessed by Cell Production Team.

4. Verification and authentication

4.1. Ethics/consents

Ethics approval for the project ('Development of human embryonic stem cells from excess ART embryos') was obtained from the Genea Ethics Committee on 21 February 2001. Excess ART embryos were fully consented for stem cell derivation by all responsible people through an informed consent process (signed de-identified consent form can be provided upon request). Donors have received no payment or financial benefits for their donation. Genea043 has been derived from a donated, fully commercially consented human embryo, originally created by assisted reproduction technology (ART) for the purpose of procreation, under Australian National Health and Medical Research Council (NHMRC) licence 309703. This licence was issued to GENEA on 16 April 2004. More information about the licence can be obtained from the NHMRC webpage at http://www.nhmrc.gov.au/health-ethics/human-embryos-and-cloning/database-licences-authorising-use-excess-art-embryos.

Table 1
CGH analysis summary of Genea043 (Passage 10, 2 enzymatic) reporting a male cell line and no abnormalities detected.

	CGH summary
Sample name	Genea043p10_2
Date reported	20th August 2013
Hybridisation balance	A balanced hybridization was observed for all chromosomes, relative to reference DNA
Copy number change	No copy number changes >400 kb were detected
Interpretation	Male cell line — no abnormalities detected

Table 2
STR profile of Genea043 (Passage 3), demonstrating male allele pattern.

	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
SIVF043_p3	13,14	30,30.2	8,10	12	16,17	7,8	11,13	11	16,18	13,14	14,17	8	15,16	11,13	23

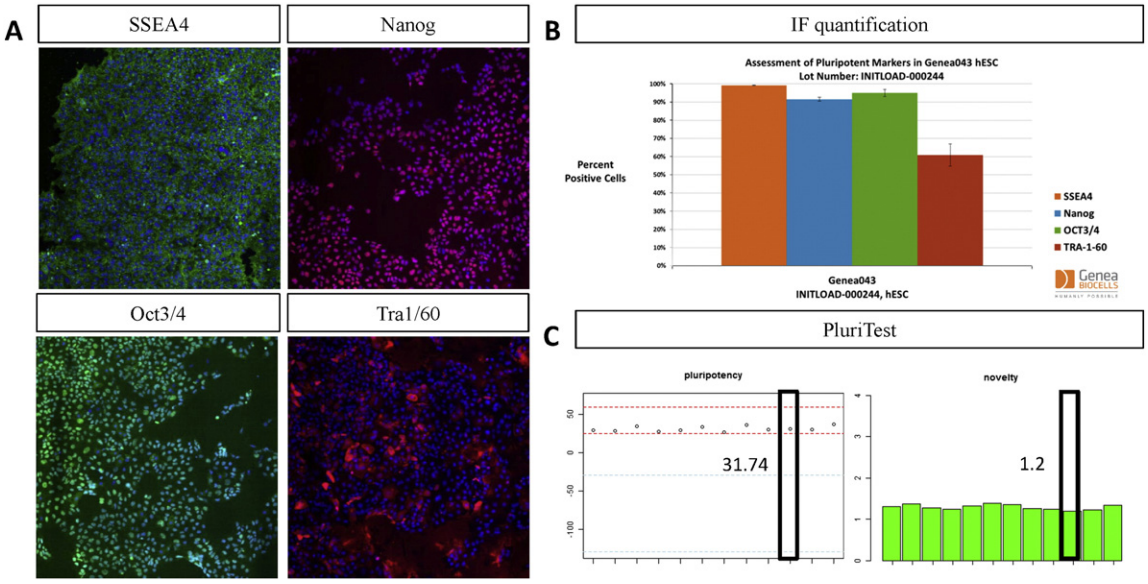

Fig. 2. Pluripotency validation of Genea043. A) Immunofluorescent staining (10×) of pluripotent cellmarkers SSEA4 (green), Nanog (red), Oct3/4 (green) and Tra1/60 (red), Passage 10 (2 enzymatic). B) Quantitation of expression of pluripotent markers. C) PluriTest pluripotency (left) and novelty (right) scores with Genea043 (Passage 11, 3 enzymatic) outlined in black.

4.2. Morphology

The derived stem cell line, Genea043, morphologically displays adherent monolayer of compact cells in well-defined colonies with high nuclear to cytoplasmic ratio and prominent nucleoli (Figs. 1A/B).

4.3. Genetic analysis

The cell line has been karyotyped (Fig. 1C, Supplementary Fig. 1) and tested by CGH (Table 1, Supplementary Fig. 2), which demonstrated 46, XY karyotype, consistent with original derivation. Analysis of STR markers showed Allele pattern consistent with male genotype Table 2, Supplementary Fig. 3).

4.4. Disease status

Unaffected.

4.5. Pluripotency

GENEA043 is pluripotent by:

1. Alkaline Phosphatase stain positivity (Fig. 1B)
2. Immunofluorescence with 92% Nanog positive, 95% Oct4 positive, 61% Tra1–60 positive, and 99% SSEA4 positive (Fig. 2A, quantified in 2B)
3. PluriTest with a 31.74 Pluripotency score and 1.2 Novelty score (Fig. 2C).

4.6. Sterility

The cell line is tested and found negative for Mycoplasma and any visible contamination (Supplementary Fig. 4).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2015.11.010.

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